Virological studies in Reiter's disease

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There is strong presumptive evidence that Reiter's disease (RD), as it occurs in Britain and North America, is related to a transmissible disease of venereal origin (Maddocks, 1967; *British Medical Journal*, 1969). There have been several reports of attempts at isolating a responsible organism, but no generally accepted causative agent has been found. In recent years claims have been made that mycoplasmas (Klieneberger-Nobel, 1959; Csonka, Williams, and Corse, 1966) or *Chlamydia*-like organisms (Jones, Collier, and Smith, 1959; Siboulet and Galistin, 1962; Gear, Gordon, Jones, and Bell, 1963; Schachter, Barnes, Jones, Engleman, and Meyer, 1966) are aetiologically associated with RD or the related condition non-specific urethritis (NSU). In the case of mycoplasmas, these claims have not withstood critical analysis (Black and Rasmussen, 1968; Taylor-Robinson, Addey, Hare, and Dunlop, 1969), whereas the evidence for a causative association of *Chlamydia*-like organisms with a proportion (perhaps 40 per cent.) of cases of NSU (though not of RD) is becoming more generally accepted (Dunlop, Jones, Darougar, and Treharne, 1972). Even if this evidence is correct, the aetiological agent or agents responsible for RD and for the majority of cases of NSU remain unidentified.

In addition to these studies, there have been several negative reports of attempted virus isolations in RD and NSU (Csonka and Furness, 1960; Whittington, 1962; Ford, 1968). Most of these reports deal with studies of urethral washings and there are only a few papers concerning studies of RD synovial fluid (SF) (Ford and Rasmussen, 1964; Claus, McEwen, Brunner, and Tsamparlis, 1964; Ford, 1968). I could find no reports of the inoculation of mice with RDSF.

Results are given here of attempts at virus isolations in tissue culture, and in suckling and immunosuppressed mice, from synovial fluid from two cases of RD.

Material and methods

SYNOVIAL FLUIDS

All specimens were obtained by aspiration under sterile conditions of effusions of knee joints.

From Case 1 (Reiter's disease) synovial fluid specimens SF1, SF2, and SF3 were obtained at weekly intervals during the initial attack. Tetracycline 250 mg. four times a day was started after SF2 was obtained. Specimens SF4 and SF5 were received one week apart during a relapse, 6 months after the initial illness.

From Case 2 (Reiter's disease) specimens SF6 and SF7 were obtained 7 weeks apart during two separate episodes, 12 and 14 months respectively after the original gynaecological illness (see *Case Reports*).

SF8 and SF9 were obtained from two separate cases of osteoarthrosis with bacteriologically sterile knee joint effusions.

Specimens, as far as possible, were inoculated directly into tissue culture tubes and/or mice within 15 to 60 min. of being obtained from the patient. Material not used immediately was divided into small aliquots and stored at −70°C.

MICE

Outbred TO mice, bred by the Animal Division, Clinical Research Centre, were used for all experiments. Suckling mice were inoculated with 0·02 ml. of test material intracerebrally, and/or 0.03 ml. intraperitoneally, within 48 hrs, and usually within 24 hrs, of birth. For most experiments suckling mice of the various litters available, before inoculation, were pooled and randomly distributed and returned to the mothers, who almost invariably accepted them.

In assessing results of these experiments, 'early' deaths (defined as deaths occurring within 7 days of inoculation and presumed to be due to trauma associated with the procedure) have been disregarded. Over 90 per cent. of these early deaths took place within 4 days, and in an average experiment affected 5 to 10 per cent. of inoculated mice.

Immunosuppressed mice were prepared as follows. 4-week-old male mice were thymectomized and 4 weeks later they were irradiated with an 850 r dose and reconstituted the same day by intravenous inoculation of 5×10^6 autologous bone marrow cells obtained by aspiration of femurs of killed TO mice. Untreated male

mice of the same age were used as controls. The mice were used in experiments 3 weeks after irradiation and reconstitution.

TISSUE CULTURES

Primary monkey kidney (MK) material was obtained from the M.R.C. Laboratories, Hampstead (early experiments), trypsinized, and put up into basic Eagle's medium with 10 per cent. foetal calf serum ('growth medium') until the cells were confluent, when the medium was changed to Eagle's with 0·2 per cent. bovine plasma albumin (BPA), penicillin 100 u./ml. and streptomycin 100 μg./ml. ('maintenance medium'). Because it was found that this tissue was frequently infected with SV40 ('foamy') virus, prepared tubes were obtained from Flow Laboratories Ltd., Irvine, Scotland, for later experiments. These cells were supplied in Melnick B medium and were washed on receipt and changed to maintenance medium.

MRC5, L132, and W138 cells were stock laboratory cell lines grown in Eagle's medium with 10 per cent. calf serum, and maintained during virus isolations in Eagle's medium with 0·2 per cent. BPA.

For inoculation of SF specimens, in all cases 0·2 ml. SF was added to 1 ml. medium. Media were changed weekly. Tubes were incubated, rolling, at 33°C. (all early experiments, including all experiments giving positive results) or 36°C. (some later experiments).

For echovirus challenge, MK tubes were inoculated in the usual way with stored SF specimens and incubated at 33°C. 12 days later, at which stage no cytopathic effect (CPE) was discernible, 0·2 ml. of echovirus-11, prediluted to 1,000 TCD_{50} was added (Day 0) and the tubes further incubated for 3 days at 36°C. The tissue culture fluids were harvested on Days 1, 2, and 3 and stored at −70°C.; they were later thawed and titrated for echovirus titre by inoculating serial dilutions of the harvests into pairs of tubes of continuous MK (Vero) cells.

IMMUNODIFFUSION

5 ml. of 1 per cent. agar were poured into 5-cm. tissue culture grade Petri dishes (Nunclon). When set, the agar was punched with a central and four radial wells, each 5 mm. in diameter (central to radial well edge-to-edge distance 5 mm.). The wells were filled with serum or SF and incubated in humidified boxes at either 37°C. or room temperature, and examined from time to time for the development of precipitin lines.

Case reports

Case 1, a male carpenter aged 23, was admitted on August 12, 1971, with urethral discharge and dysuria for 6 days, sore red eyes for 4 days, and pain and swelling of the left knee for 2 days. On admission he had a fever of 38·5°C., bilateral conjunctivitis, and a massive painful effusion of the left knee joint. The fever and urethral discharge settled within a week, but he developed a large corneal erosion due to keratitis, and the knee effusion required tapping on three occasions each a week apart.

At each aspiration about 60 ml. turbid yellow fluid was withdrawn. This synovial fluid contained approximately 1,000 cells/μl., with 60 to 80 per cent. polymorphs, and was bacteriologically sterile, as were specimens from the conjunctivae and urethra. Investigations for gonorrhoea and serological tests for syphilis were negative. Complement-fixing antibody titres against the psittacosis-lymphogranuloma venereum group of organisms were <1:10 for both acute and convalescent sera (test performed by the Public Health Laboratory Service, Middlesborough). The erythrocyte sedimentation rate reached a peak of 90 mm./1st hr 4 weeks after admission.

Courses of phenylbutazone and tetracycline were given. He was discharged 6 weeks after admission when he still had moderate pain but no effusion in the left knee. He was clinically cured 4 months later, and by December, 1971, the erythrocyte sedimentation rate had returned to a normal level of 4 mm./1st hr. However, on February 10, 1972, 6 months after the initial presentation, he relapsed with a recurrence of all symptoms. The erythrocyte sedimentation rate was 77 mm./1st hr. On this occasion the joint effusion was aspirated twice. Tetracycline and phenylbutazone were again given and the patient was discharged on February 22, 1972, when he was well apart from pain in the right ankle.

Case 2, a female dancer, aged 21, was initially admitted in December, 1970, when a bilateral tubo-ovarian abscess was drained. The pus was bacteriologically sterile. On December 31, 1971, she was readmitted for 1 week with bilateral knee joint effusions, which were tapped; cloudy fluid was withdrawn with 6,800 white cells/μl. (44 per cent. polymorphs, 56 per cent. mononuclears), and a synovial biopsy showed a moderate round cell infiltrate with scanty plasma cells. She had also noted pain in the proximal interphalangeal joint of her left index finger.

She was again admitted on February 1, 1972, because of recurrent pelvic symptoms and was submitted to laparotomy and drainage of a tubal abscess. Shortly afterwards she developed a recurrence of the right knee effusion and this was again tapped. All pus and SF specimens were bacteriologically sterile. Serological tests for syphilis were negative, but the complement-fixing antibody titre against the psittacosis-lymphogranuloma venereum group of organisms determined on a serum taken in December, 1971, was 1:640.

By May, 1972, the patient was asymptomatic.

Results

TISSUE CULTURE EXPERIMENTS

A CPE developed on each occasion when SF taken during the initial presentation of Case 1 was inoculated directly into MK tissue culture tubes (specimens SF1, SF2, and SF3: see *Materials and methods*). The CPE was noted between 3 and 5 days

after inoculation in paired tubes to which SF1 or SF2 were added and at 13 and 31 days in the case of SF3 (Fig. 1).

Successful passage of CPE was obtained for up to two generations when fresh tubes were inoculated directly with medium from tubes seeded 7 to 21 days
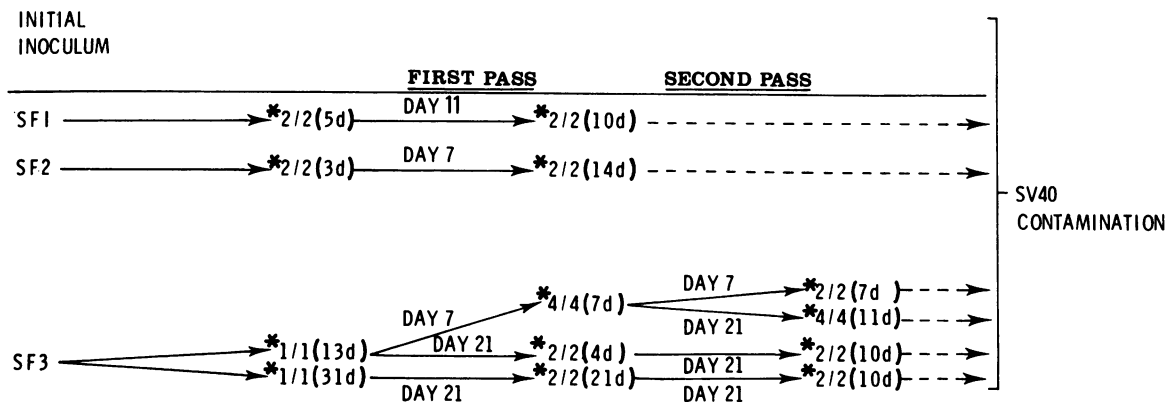
INITIAL
INOCULUM

		FIRST PASS	SECOND PASS	
SF1	→*2/2(5d)	DAY 11 →*2/2(10d)	-----------------→	
SF2	→*2/2(3d)	DAY 7 →*2/2(14d)	----------------→	SV40 CONTAMINATION

SF3 ──→*1/1(13d)
     ──→*1/1(31d)

DAY 7 ──→ *4/4(7d) ── DAY 7 ──→ *2/2(7d) }- - - →
                              DAY 21 ──→ *4/4(11d)- - - →
DAY 21 ──→ *2/2(4d) ── DAY 21 ──→ *2/2(10d)- - - →
DAY 21 ──→ *2/2(21d) ── DAY 21 ──→ *2/2(10d)- - - →

FIG. 1   *Details of passage experiments with fresh material in monkey kidney tissue. *Number of tubes showing CPE/number inoculated. Time to first recognition of CPE is shown alongside (d=days)*
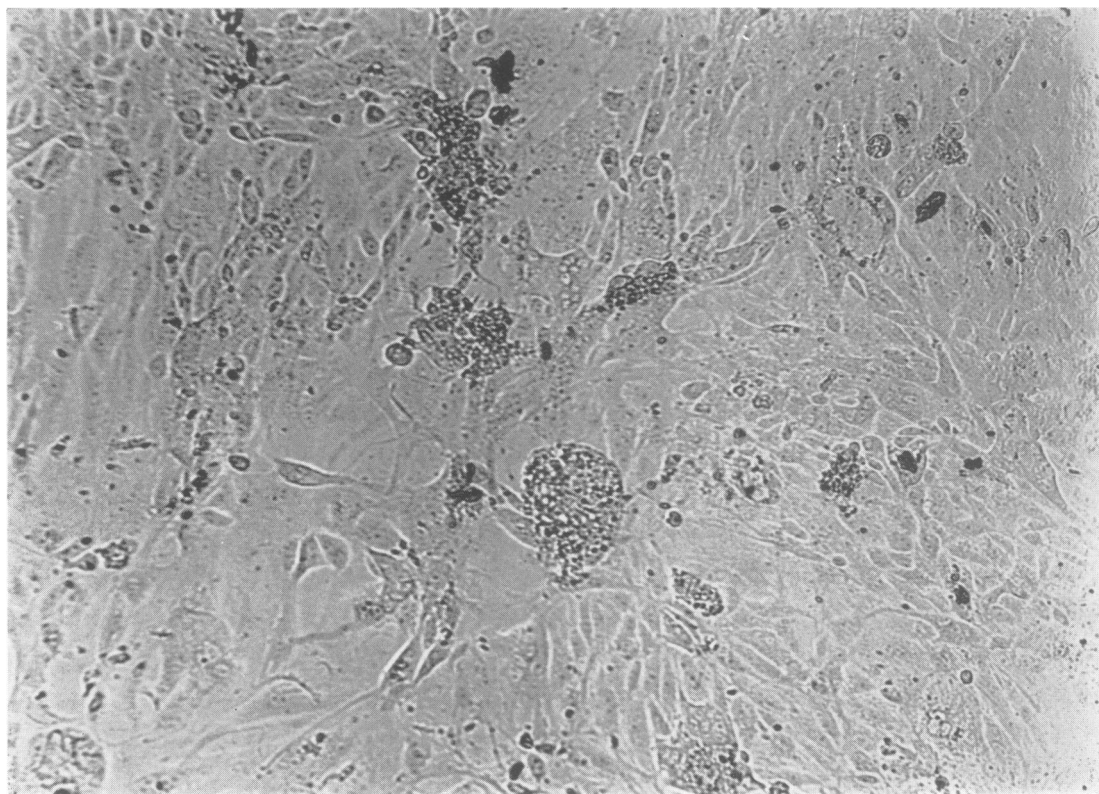

FIG. 2(a)   *CPE in monkey kidney tissue in a tube inoculated 14 days previously with medium on first passage after primary inoculation with SF2.   ×200*
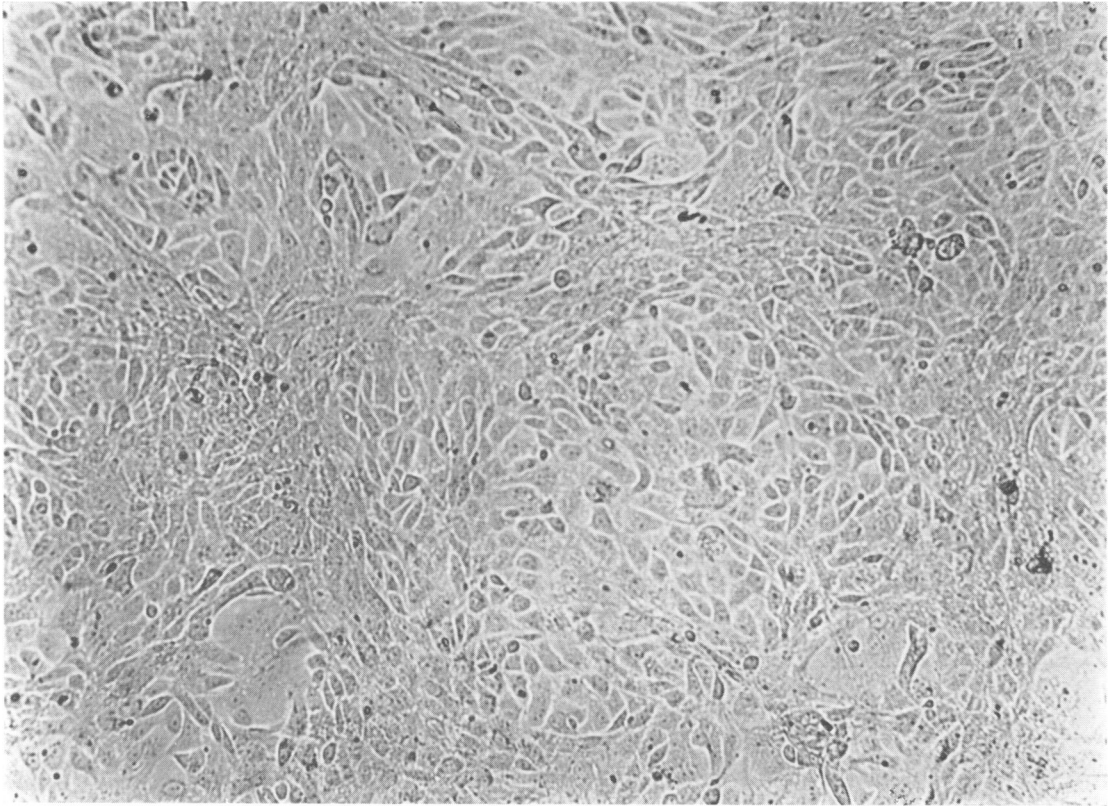
F I G . 2(*b*)   *Uninoculated monkey kidney tissue of same age.*   ×200

previously with each of the three specimens SF1, SF2, or SF3. The CPE obtained in these cases was identical to the original CPE, and became apparent between 4 and 14 days after inoculation in all 22 tubes used in these direct-passage experiments. Unfortunately, owing to contamination of our MK tubes with SV40 virus, it was impossible to interpret results of further attempts at direct passage.

The photographs (Fig. 2) show a typical CPE (in this case in a tube in the first passage after primary inoculation with SF2), with an uninoculated control culture of similar age for comparison.

The CPE could not be reproduced when the above specimens (SF1, SF2, and SF3, and media from MK tubes showing CPE) were tested after storage, *i.e.* after freezing and thawing. Nor did any CPE develop after inoculation of MK tubes with SF specimens from the same case in relapse (SF4 and SF5), from Case 2 in relapse (SF6 and SF7), or from cases of osteoarthrosis (SF8 and SF9), even when these specimens were inoculated directly and without freezing.

On two occasions, tests of frozen SF1 and SF2 and of frozen media from MK tubes showing CPE were made alongside fresh SF3 and fresh positive MK media, with differential effects—*i.e.* a CPE appeared in tubes inoculated with fresh but not with frozen material. Therefore, it seems unlikely that these results can be entirely explained by varying sensitivities of different batches of MK cells, even though it was unfortunately not possible to carry out any other such simultaneous experiments.

Results of primary inoculation of MK tissue culture tubes with SF specimens are shown in the Table (overleaf).

Fig. 1 gives details of passage experiments with SF1, SF2, and SF3.

Slides prepared from tubes with CPE were stained and examined for the presence of inclusion bodies No inclusions were found.

When L132 and W138 cells were inoculated with some acute RDSF specimens, generalized degenerative changes occurred in 5 to 7 days. However, as these changes were indistinguishable from those

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TABLE   *Primary inoculation of tissue culture tubes and of mice with synovial fluids*

Details of inoculum					Tissue culture	Mice				Affected mice	
Patient No.	Disorder	Stage	Synovial fluid	State of specimen	CEP in MK tubes	Age	Route of inoculation	Affected litters		Illness	Death
1	Reiter's disease	Acute	SF1	Fresh	2/2ᵃ (5 days)	—					
				Frozen	0/2	—					
			SF2	Fresh	2/2 (3 days)	—					
				Frozen	0/8	Suckling	i.c. only	3/3ᵇ		26/28ᶜ	2/28ᶜ
						Suckling	i.p.±i.c.ᵈ	0/2		0/15	0/15
			SF3	Fresh	2/2 (13 and 31 days)	—					
				Frozen	0/18ᵉ	Suckling	i.c. only	4/9ᶠ		26/74	10/74ᵍ
						Suckling	i.p.±i.c.ᵈ	0/2		1/13	1/13ᵍ
						Adult	i.p.+i.c.			1/22	1/22
						Adult-IS	i.p.+i.c.			0/6	0/6
		Relapse	SF4	Fresh	0/4	—					
				Frozen	0/34ᵉ	Suckling	i.p.+i.c.	0/9ʰ		0/51	0/51
						Adult-IS	i.p.+i.c.			0/7	0/7
			SF5	Fresh	0/6	Suckling	i.p.+i.c.	0/2		0/9	0/9
				Frozen	0/12ᵉ	—					
2	Reiter's disease	Relapse	SF6	Fresh	0/6	Suckling	i.c. only	1/2		8/18	8/18ⁱ
				Frozen	0/6	—					
			SF7	Fresh	—	Suckling	i.p.+i.c.	0/2		0/11	0/11
3	Osteoarthrosis		SF8	Frozen	0/10ᵉ	Suckling	i.p.+i.c.	0/2		0/15	0/15
4	Osteoarthrosis		SF9	Frozen	0/22ᵉ	Suckling	i.p.+i.c.	0/4		2/27	2/27ʲ
						Adult	i.p.+i.c.			1/25	1/25
						Adult-IS	i.p.+i.c.			0/6	0/6ʲ
—	CPE-positive MK medium			Frozen		Suckling	i.p.+/or i.c.	0/6		2/44	2/44ⁱ
—	Control mediumᵏ			Frozen		Suckling	i.p.+/or i.c.	0/17		2/119	2/119ʲ

i.c. =intracerebral; i.p. =intraperitoneal; IS =immunosuppressed.
ᵃNumber of tubes showing CPE/number inoculated. Time from inoculation to first recognition of CPE appears in brackets.
ᵇNumber of litters with >1 affected mouse/number of litters inoculated. Mice were observed for 40 days after inoculation.
ᶜNumber of mice affected/number inoculated. Mice dying within 7 days of inoculation have been discounted. Except for solitary runts (footnotes g and j), deaths occurred 14 to 20 days after inoculation.
ᵈOne litter inoculated i.p. and i.c., one litter i.p. only.
ᵉIncludes some tubes inoculated with diluted synovial fluid (1:10 or 1:100). Also, for SF4 and SF9, six tubes each were incubated at 36°C. instead of the usual 33°C.
ᶠIncludes two litters (16 mice) inoculated with diluted (1:20) synovial fluid. No illness or deaths occurred in this group.
ᵍIncludes a solitary runt in an otherwise healthy litter (not counted as an affected litter—see footnote b).
ʰIncludes six litters inoculated with diluted synovial fluid (2 litters each 1:10, 1:100, 1:1000).
ⁱIn this group 8/8 of one litter and 0/10 of a simultaneously inoculated litter were affected (see text).
ʲIncludes two solitary runts in two otherwise healthy litters (not counted as affected litters—see footnote b).
ᵏIncludes sterile saline, Hanks's balanced salt solution, and CPE-negative MK medium.

seen in uninoculated tubes 7 to 10 days later, and presumed due to 'ageing' of the cells, they were not pursued.

MRC5 cells appeared unaffected by inoculation of SF specimens.

In echovirus challenge experiments there was no convincing interference, on two occasions, with the growth of echovirus-11 in tubes previously seeded with stored SF3 and SF4 compared with previously uninoculated tubes.

MOUSE INOCULATION EXPERIMENTS
In several experiments in which suckling mice were inoculated intracerebrally with stored specimens from the acute stage of illness of Case 1, a number became ill, usually between 12 and 20 days after inoculation, and some died. The illness consisted of poor development, lethargy, ruffled fur, and sometimes incoordination and discoloration of fur at the nape of the neck.

The Table gives details of the effect of primary inoculation of mice with SF and other materials. It can be clearly seen that a significant number of suckling mice inoculated intracerebrally with SF2 and SF3 became ill, a total of 52 out of 102 (51 per cent.) of mice so inoculated being affected. If those

given intraperitoneal injections are included, illness occurred in 53 out of 130 (41 per cent.), still a considerably higher proportion than in other groups. In comparison relapse SF (specimens SF4, SF5, SF6, and SF7) affected only eight of 89 animals (9 per cent.), and as all these eight mice came from one litter only it seems at least possible that they succumbed to an unrelated illness. With osteoarthrosis SF (SF8 and SF9), only two suckling mice of 42 (5 per cent.) became ill, and both these mice were solitary runts in otherwise healthy litters. Of mice given MK medium and other control fluids, four of 163 (2 per cent.) became ill; again all four were solitary runts.

Attempts to passage illness by intracerebral inoculation of litters of suckling mice with fresh brain suspensions prepared in Hanks's balanced salt medium (BSS) from mice inoculated 14 to 21 days previously with SF specimens have met with some success. Four of 66 (6 per cent.) mice given 10 to 20 per cent. brain suspensions from SF2- or SF3-inoculated animals became ill; six of eighteen (33 per cent.) given 1 per cent. suspensions were affected. No illness developed in control litters given 10 or 1 per cent. brain suspensions prepared from mice previously inoculated with BSS.

Histological examination of haematoxylin and eosin stained slides of brains and other organs of affected animals did not reveal the presence of any distinctive pathological feature.

No illness developed when immunosuppressed or normal adult mice were inoculated with SF specimens. Nor was there any obvious enhancement of illness development when suckling mice were injected with hydrocortisone, 1 mg. intraperitoneally on alternate days, commencing on the fifth day after SF inoculation.

IMMUNODIFFUSION

Acute and convalescent sera of Case 1, convalescent serum from Case 2, and a number of sera from normal subjects were tested against SF specimens SF3, SF7, and SF9. No precipitin lines developed on any plate.

Discussion

There have been no previous reports of attempts to culture RDSF on monkey kidney tissue, or of inoculation of mice with this material. The nearest approach has been that of Claus and others (1964) who cultured synovial biopsy tissue from two cases of RD on monkey kidney tissue, without effect. Ford and Rasmussen (1964) mentioned attempts to demonstrate a virus aetiology of RD by inoculation

of 'urethral, synovial, or conjunctival exudate . . . [into] . . . HeLa, Chang conjunctival, human amnion, monkey kidney, and human synovial cell cultures'; it is not stated specifically whether SF was inoculated into monkey kidney cell cultures.

Urine and urethral specimens from cases of RD and NSU have been cultured on monkey kidney cells, and in eggs and other tissues, without demonstrating any CPE (Csonka and Furness, 1960; Ford, 1968).

There have also been negative reports of the effects of inoculating RDSF into tissue culture material of human amnion (Claus and others, 1964; Ford, 1958), Chang conjunctival cells (Ford, 1958), and human 'synovial' cells (Ford, 1968).

Ford (1968) also described unsuccessful attempts to demonstrate 'interference' in monkey kidney cell cultures inoculated with urethral washings from NSU and subsequently challenged with echovirus-9.

Several authors, reporting unsuccessful virus cultures in RD and NSU have commented on the importance of inoculating culture material without delay (Morton, Gillespie, and Wilson, 1964) and on the possible deleterious effect of storage at $-70°C$. (Whittington, 1962). The last author reported studies of urethral washings of twenty cases of NSU, including one of RD; however, the RD specimen was frozen before testing. In her experiments, a CPE passageable for two generations was seen in HeLa cells inoculated with material from one case of NSU.

Our results, showing that a CPE developed in tissue culture only when the material was freshly inoculated and was obtained from the acute stage of the disease, emphasize the importance of these two points. In the case of rheumatoid arthritis it has long been suspected that the disease symptomatology is the result of a disturbance of the immune response to a previous synovitis caused by an as yet unidentified organism, and that the organism itself may well not be present, or may be present only in minute amounts, during the later stages of the disease. There is some experimental evidence to support this idea (Webb, Ford, and Glynn, 1971). The relapses of RD might have a similar aetiological basis and this has recently been proposed in relation to post-dysenteric Reiter's syndrome (Larsen, 1971). If so, it would explain our failure to obtain a CPE using relapse material. On the other hand there have been reports that an agent in chronic rheumatoid arthritis synovial tissue may be transmitted to newborn mice and rats by intraperitoneal injection or ingestion, resulting in vascular lesions in the extremities of these animals (Warren, Marmor, Liebes, and Rosenblatt, 1972).

That mice became ill only when they were inoculated intracerebrally and within 48 hrs of birth is

analogous to the effects of coxsackie virus *in vivo* (Grist, Ross, Bell, and Stott, 1966). In our few successful passage experiments, 1 per cent. brain suspensions were more likely to be effective than 10 per cent. and this may reflect the presence of an inhibitor in the concentrated tissue suspension. This dilutional effect should be borne in mind in any future experiments.

It cannot be certain that the effects demonstrated here were due to a virus. However, penicillin and streptomycin were routinely included in the media and, of the two other groups of organisms suspected in the aetiology of RD, *Chlamydia* are uniformly inhibited by penicillin (Davis, Dulbecco, Eisen, Ginsberg, and Wood, 1969) and some species of mycoplasma are inhibited by streptomycin, including in particular the species that have been isolated from human genital tracts and reputedly from joints— *M. hominis*, *M. fermentans*, and T-strains (Braun, Klein, and Kass, 1970). That either of these agents was responsible for our results would, therefore, seem most unlikely.

One must also consider the possibility that the results obtained here may have been caused by non-specific toxic components of the SF. This cannot be entirely excluded, particularly in relation to the mouse experiments, though the apparent enhancement of illness development through use of diluted tissue suspensions in passage experiments, and the absence of effects after inoculation of relapse RDSF and of osteoarthritic SF, makes this possibility less feasible. In addition, it seems unlikely that a toxic effect could survive two passes in tissue culture, especially as on several occasions (passes made at 21 days, Fig. 1) the tubes underwent two changes of medium before successful passage.

As several batches of MK cells were contaminated with SV40 virus, one must ask if the CPE described in these experiments was merely an unusual form of the effect of this agent. This too seems unlikely, as the CPE (Fig. 2) was quite different from that of SV40; and SV40 contamination was not seen in other MK tubes in the batches in which a CPE developed, whether the tubes were uninoculated or (in some instances—see *Results*) inoculated with frozen and thawed SF or MK medium.

The criticism might be made that Case 2 does not fulfil the classical criteria of RD (Reiter, 1916), although no other diagnosis seems more likely (*British Medical Journal*, 1971). However, Case 1, which provided the material producing positive results, satisfies the strictest requirements for the diagnosis (Weinberger, Ropes, Kulka, and Bauer, 1962).

The potential wider importance of the elucidation of the problem of RD, particularly in relation to rheumatoid arthritis, has been emphasized (*British Medical Journal*, 1969). On the basis of the results reported in this paper, it is suggested that, in future studies, material should be obtained from early cases and should be inoculated into target cells and suckling mice with very little delay and without freezing.

Summary

Synovial fluid from two cases of Reiter's disease has been inoculated into tissue culture tubes and into mice. In tissue culture, a reproducible cytopathic effect (CPE) was obtained on primary rhesus monkey kidney cells with three out of three specimens from one case, when the following criteria were observed: the material was obtained during the initial acute presentation; it was inoculated without delay; it was not frozen.

This CPE survived two passes, but the results of further attempted passage could not be interpreted because of contamination of the tissue culture cells with SV40 virus.

The same material after storage produced illness in some suckling mice 12 to 20 days after intra-cerebral injection, and there was evidence that passage by inoculation of a 1 per cent. brain suspension was successfully accomplished.

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Études virologiques dans la maladie de Reiter

SOMMAIRE

Le liquide synovial provenant de deux cas de maladie de Reiter a été inoculé en culture de tissu et chez des souris. Dans la culture tissulaire, on a obtenu un effet cytopathique reproductible (CPE) sur des cellules primaires de rein de singe rhésus dans trois des trois échantillons dans un cas, lorsque les critères suivant furent observés: que le matériel soit obtenu pendant la période aigüe, qu'il soit inoculé sans délai, qu'il ne soit pas réfrigéré.

Le CPE a pu survivre à deux passages mais les résultats de passages ultérieurs n'ont pas pu être interprétés à cause de la contamination de la culture de tissu par le virus SV-40.

Le même matériel, après conservation, a rendu malades quelques souris venant de naître, 12 à 20 jours après injection intra-cérébrale et l'on obtint la preuve que le passage par inoculation d'une suspension de cerveau à 1 pour cent peut être accompli avec succès.